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Study on the Amylolytic Activity of Bacteria Isolated from Spent Mushroom Substrate

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Seven different bacterial strains were isolated from spent mushroom substrate (SMS) and tested for starch hydrolysis. Out of them, three bacterial strains namely *Bacillus spp (B1), Pseudomonas spp, Bacillus spp (B2)* were able to form clear zone in starch hydrolyzing medium. These three strains were subjected for amylase assay and extra cellular amylase production was observed as 214,180 and 245μg/ml respectively.

Key words: SMS, Starch hydrolysis, *Bacillus, Pseudomonas*, extra cellular amylase.

Starch degrading amylolytic enzymes are of greater significance in biotechnological applications ranging from food, textile and paper industries (Pandey *et al.,* 2000).Amylase is the name given to glycoside hydrolase enzymes that breakdown starch into maltose molecules and has been derived from several sources such as plants, animals and microbes. Amylases from fungal and bacterial sources have dominated in their application in industrial sector. A large number of them are available commercially and they have completely replaced chemical hydrolysis of starch processing industries. The major advantage of using microorganisms for production of amylase is due to their ability for easy manipulation to obtain enzymes of desired characteristics.

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MATERIAL AND METHODS

Collection of sample

Spent mushroom substrate (SMS) samples for isolation of bacteria were collected in different sites of mushroom farms situated around Gandhigram Rural University, Gandhigram, Dindigul (DT), Tamil Nadu.

Isolation of Bacterial SMS

The SMS samples were collected in clean, sterilized Petri plates mixed thoroughly and subjected for bacterial isolation by standard dilution technique and spread plate method. Further the bacterial isolates were identified using morphological characteristics gram staining, motility test and biochemical tests (Cappucino *et al*., 2005)

Starch hydrolysis assay

Bacteria isolated from SMS were subjected for starch hydrolysis assay by plating the diluted samples on Lama *et al.,* (1991)isolating medium and incubating the petri dishes at 35°C for 3 days . Composition of *Lama et al.,* (1991) medium is KH_2PO_4, 3.10 g;

$(NH_4)_2$ S04,2.5 g;$MgSO_4 7H_20$,0.2 g ; yeast extract 2.0 g; starch 5.0 g and distilled water to make final volume 1000 ml. pH 7.2. Pure line cultures of the bacterial flora isolated from SMS were established on nutrient agar. Starch hydrolysis assay (Case & Johnson, 1985) using gram's iodine was performed with all the isolates to confirm their amylase production capability. The bacterial isolates that exhibited widest zone of clearance indicated their high amylase production capability was tested for extra cellular amylase production.

Extra cellular production of amylase by the potential bacterial isolates of SMS

The amylase producing potential of the isolates was carried out by inoculation 1.0% (0.1 OD) of isolate to Lama et al broth (1991) and incubated at 50° C for 120 hrs. After the production of enzyme, the cultures were centrifuged at 5300 rpm for 10min and the supernatant was tested for amylase assay.

Amylae assay was taken up using phosphate buffer (0.1m,pH 7.0); starch solution (1%) and dinitrosalicyclic acid (DNSA)reagent and standard maltose. The reaction mixture contained 0.5ml of starch solution, 0.3ml of phosphate buffer and 0.2ml of enzyme. The control was run without adding any enzyme. The tubes were incubated at 50° C for 5minutes and later 3ml of DNSA was added to each tube. The tubes were immersed in boiling water bath for 15min and after cooling the absorbance were measured at 540 nm. By drawing the standard graph; the enzyme activity which represents the amount of maltose produced in the reaction mixture per ml per unit time was calculated.

RESULTS

Bacterial colonies of spent mushroom substrate samples were recorded as 86,110 at the 10^{-5}, 10^{-6} dilutions with 178.18×10^{-5} CFU/ml. Seven different colonies were isolated and identified at genus level on the basis of biochemical tests (Table 1). The bacterial colonies isolated from SMS varied from even nature to opaque nature; their colour also varied from yellow, white to green .The bacterial isolates of SMS such as *Micrococcus* spp, *Bacillus* spp(B1), *Bacillus* spp (B2) and *Staphylococcus* spp were found to be gram positive and *Pseudomonas* spp,

Table 1. Biochemical characteristics of Bacteria isolated from SMS

Isolate code	Gram Stain	Motility test	MR Reaction	VP Reaction	Indole production	Citrate test	Starch Hydrol-ysis	Gelatin Lique-Fication	Sugar Fermen-tation	H2S Pro-duction	Catalase activity	Organisms
A	Cocci+	-	-	-	-	+	-	+	-	-	-	*Micrococcus* spp
B	Rod+	+	-	+	-	+	+	-	-	-	+	*Bacillus* spp
C	Rod-	+	-	-	-	+	+	+	-	-	+	*Pseudomonas* (B_2)
D	Rod+	-	-	-	-	-	+	+	A	-	-	*Bacillus* spp (B_2)
E	Cocci+	-	+	-	-	-	-	+	A	-	+	*Staphylococcus* spp
F	Rod-	+	+	-	+	-	-	-	AG	-	+	*E. coli*
G	Rod-	+	+	-	+	-	-	+	-	+	+	*Proteus* spp

AG-Acid Gas production**A** - Acid only, the broth has turned Yellow

E.coli, Proteus spp as gram negative. Among the seven isolates *Bacillus* spp (B1), *Pseudomonas* spp, *E.coli* and *Proteus* spp were found to be motile and the rest of the species were nonmotile.

In starch hydrolysis assay, bacterial isolates such as *Bacillus* spp (B1), *Pseudomonas* spp, *Bacillus* spp(B2) exhibited widest zone of clearance indicating their high amylase producing efficiency.

High enzyme activity which is recorded from the amount of maltose production, was 245 µg/ml in *Bacillus* spp(B2), 214 µg/ml in *Bacillus* spp (B1) and 180 µg/ml in *Pseudomonas* spp.

DISCUSSION

Introduction of amylase for the commercial purpose represents a milestone in the food, pharmaceutical and textile industries (Rajnikanth & Ravi, 1998). Commercial mushroom compost that is inhabited by wide consortium of microbes would be a new novel source to isolate microbes (Sharma *et al.,* 2006). In the present study also, spent mushroom substrate (SMS)was recorded as a potential source to isolate bacteria for exploring amylase production. Viji *et al.,* (2002) reported a number of bacterial flora like *B.subtilis, B.licheniformis, E. coli, Pseudomonas aeruginosa* from SMS. In the present study among the seven bacterial isolates of SMS *Bacillus* spp (1 & 2) and *Pseudomonas* spp were recorded their efficiency to hydrolyse starch by producing amylase. Similar results stating the production of extra cellular amylase from *Bacillus subtilis, B. licheniformis* were recorded by Yoneda (1974). Sharma & Khosla (2002) have reported that there is a good correlation between amylase forming activity and tendency to lyse. The activation of autolytic enzymes near the cellwall supposedly takes place when growth and oxidative metabolism slows down during the stationary phase; apparently,this permits the liberation of extracellular amylase. This study shows that SMS can be explored as a new habitat for isolating potent enzyme producing bacterial strains.

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